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PREVALENCE AND RISK FACTORS OF MULTIDRUG RESISTANT AND CARBAPENIMASE PRODUCINGENTEROBACTERIACAE AMONG PATIENTS WITH URINARY TRACT INFECTION AT THE UNIVERSITY OFGONDAR HOSPITAL, NORTHWEST ETHIOPIA

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LIST OF ABBREVIATIONS

CFU	Colony Forming Unit
CPE	Carbapenemase Producing Enterobacteriaceae
ESBL	Extended Spectrum β-Lactamase
EARS-Net	European Antimicrobial Resistance Surveillance Network
ICU	Intensive Care Unit
IMP	Imipenemase
KPC	*Klebsiella pneumoniae* Carbapenemase
MDR	Multi-Drug Resistant
MDRE	Multi-Drug Resistant Enterobacteriaceae
NDM- 1	New Delhi Metallo-beta-lactamase
OXA-48	Oxacillin-hydrolzying metallo-β-lactamases
UTI	Urinary Tract Infection
VIM	Verona Integron encoded Metallo-beta-lactamase

ABSTRACT

Background: Increased burden of multidrug resistant Enterobacteriaceae (MDRE)causing urinary tract infection (UTI)compounded by harboring carbapenemase producing strainsbecomes a serious threat to public health.Carbapenemase producing Enterobacteriaceae (CPE)expresses enzymes thatcan break down carbapenems. Prevalence of MDRE in different part of the world is increasing, but data about the incidence of CPE in is not yet documentedin Ethiopia.

Objective: The aim of the study was to assess the prevalence and risk factors of MDR and CPE among patients with UTIs.

Methods:A cross sectional study was conducted among 442 symptomatic UTI suspected patients atthe University of GondarHospital from February to May 2014. Systematicrandom sampling techniquewas used to select the participants. Data onsocio-demographiccharacteristics, clinical informationand possible risk factors werecollected using structured questionnaire. Mid-stream urine samples were collected and processed to characterize bacterial isolates. Disk diffusion method was used to determine the antibiotic susceptibility patterns of isolates. In this particular study, CPE isolates were detected usingCHROMagar KPC medium.Data were entered and analyzed using SPSS version 20. P-value <0.05 were considered as statistical significant.

Results: A total of 442 patients with mean age of 37.1 years were included in this study and the majorities were females (63.8%). From 183 (41.4 %) of patients, 183 Enterobacteriaceae isolates were identified; of which,160 (87.4%) were MDRE;the principalisolates were *E.coli*and*K. pneumoniae*.Moreover, 5 (2.73%) of isolates were found to be carbapenemase producers, namely *E.coli* (2), *K. pneumoniae* (2), and *E. aerogenes* (1). Significant drug resistances were observed among CPE compared to other MDRE, low resistance rates were noted to ciprofloxacin (20%). Being female (OR 4.46; P = 0.018), age (OR 1.08; P = 0.001), hospitalization(OR 5.23; P = 0.006), and prior antibiotic use (OR 3.98; P = 0.04) were associated risk factors with MDRE.

Conclusion and recommendations: Increased prevalence of MDRE and incidence of CPE were indicated in this study. Attributingrisk factors for MDRE were found to be sex (female), age, hospitalization, and history of antibiotic therapy. Therefore, efforts should be directed to reducepatient hospital stay and to maximize rationaluse of drugs. Additional and vigorous investigation especially on CPE should be encouraged.

Key words: Carbapenemase, Enterobacteriaceae, Multidrug resistant, Urinary tract infection

1. INTRODUCTION

1.1. Background

Urinary tract infection (UTI) is the presence of bacteria (bacteriuria) in urine and witha growth of a single pathogen $\geq 10^5$ colony forming units (cfu)/ml from a properly collected mid-stream urine sample. Symptoms of UTIsare dysuria, urgency and frequent urination, along with malodorous and/or cloudy urine. Signs of infection include the presence of blood (hematuria) or white blood cells (pyuria) in urine (1).

Urinary tract infections are one of the most common infectious diseases ranking next to upper respiratory tract infection. Urinary tract infections are often associated with significant morbidity and mortality. Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion dollars(2). In developing countries, including Ethiopia, the facilities for urine culture and antimicrobial susceptibility testing are still not sufficiently available, leading to improper diagnosis and irrational antibiotic treatment of UTI, which expedites the emergence of multidrug resistant (MDR) strains (3).Gram negative bacteria, especially the family Enterobacteriaceaeare the common cause of both community and hospital acquired UTIs. *Escherichia coli* and*Klebsiella pneumoniae* are most commonlyimplicated amongpatients with UTI(4, 5).

Enterobacteriaceaespread easily between humans by hand carriage as well as contaminated food and water and have a propensity to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons, which are most important factors, for emergence of MDRamong these bacteria. Theincrease rate of antibiotic resistance among Enterobacteriaceae has posed challenges in choosing empiric regimens, especially when the infections are caused by multidrug resistant Enterobacteriaceae(MDRE)(6).

Previously, the emergences of MDR among Enterobacteriaceaewere mainly due to the production of enzymes, such as pencillinases, cephalosorinases, andextended spectrum β-lactamase (ESBL). However, recently carbapenemase production is one of the main mechanisms in the occurrence of drug resistance in the family ofEnterobacteriaceae.Carbapenemase

producing Enterobacteriaceae (CPE) are a family of organisms that are difficult to treat because they have high levels of resistance to antibiotics. *Klebsiellapneumoniae* and *E. coli* are members of Enterobacteriaceae, which capable of break down all β-lactam agents including carbapenems and make it ineffective. Carbapenem such as imipenem, meropenem, ertapenem, &doripenem are considered as the last resort antibiotics to treat ESBL producing Enterobacteriaceae(7, 8).

A large variety of carbapenemases has been identified in Enterobacteriaceaebelonging to 3 classes of β-lactamases: the Ambler class A, B and D. In class A, *Klebsiella pneumoniae*carbapenemase (KPC) are encoded by the bla_{KPC} gene, which is located within Tn3-type transposons, Tn4401, and is capable of inserting into diverse plasmids of gram negative bacteria, Enterobacteriaceae in particular. In class B, Metallo-beta-lactamases (MBL) includes Imipenemase(IMP), Verona integron encoded metallo-beta-lactamase (VIM) and the recently described New Delhi metallo-beta-lactamase (NDM- 1). Imipenemase, VIM and NDM-1 are encoded by genes located within integron elements, capable of inserting in to various types of plasmid. In class D, Oxacillin hydrolyzing metallo-β-lactamases (OXA-48) encoded by bla_{OXA-48}, which shows relatively low carbapenem resistance(6).

Infections caused by CPEmost commonly occur among patients who are receiving treatment for other conditions. Patients whose care requires devices like mechanical ventilators, urinary catheters, or intravenous catheters, and patients who are taking long courses of certain antibiotics are most at risk for infections caused by MDR strains.likewisepatients admitted to critical care units for treatment of acute emergencies and chronic diseases are especially vulnerable to get these infections because of the presence of MDR strains in the environment and selective pressure on them due to overuse of antibiotics(9).

1.2. Statement of the problem

Multidrug resistantis now emerging increasingly in Enterobacteriaceae*;* this is mainly due to combined effect of drug resistance mechanisms, such as pencillinases, cephalosporinases (ESBL), and carbapenemases. Following spread of MDR strains,especiallyESBL producing Enterobacteriaceae, which can hydrolyzealmost all β-lactam drugs except carbapenem, drivesenhanced consumption of carbapenems and other broad spectrum antibiotics, which in turn promotethe occurrence of new drug resistance mechanisms. As a result, due to the occurrence of

selective pressure carbapenem resistant strains mainly mediated by carbapenemase productionemergedacross the globe.The current and extensive worldwide spread in MDRE is an important source of concern since these carbapenemases producing strains capable of break down both β-lactams and non-β-lactam drugs(10, 11).

Currently, increased burden of MDRE causing UTI compounded by harboring carbapenem resistance genesmainly among E. *coli* and *K. pneumoniae*increasingly emerged(6).These strains become a serious threat to public health, associated with high mortality rates and have the potential to spread widely. Infections are difficult, and in some cases impossible to treat and have been associated with mortality rates up to 50%. Due to the movement of patients throughout the health care system, if CPE is a problem in one facility, then typically they are a problem in other facilities in the region as well.Carbapenemase producing Enterobacteriaceae are mostly endemic in specific geographical regions, but reports of their spread into other geographical locations are point of grave concern these days(12).

Carbapenemase producing Enterobacteriaceaeincreasingly reported in different part of the world. Class A includes KPC is clinically and epidemiologically the most important enzyme. *Klebsiella pneumoniae* carbapenemase majorly isolated among nosocomial *K. pneumoniae* isolates, but they have been reported in other Enterobacteriaceaeisolates. Plasmid encoded KPCs, first demonstrated in 2001 in North Carolina, subsequently become endemic in many states of USA. Moreover, it rapidly disseminated to different countries like, France, Israel, Greece, Colombia, and china. Outbreak of KPC also documented in many European countries, South America, India. At the first time the outbreak of KPCs also reported in a few African countries, such as South Africa, Nigeria(6, 13).

In class B, IMP and VIM were first identified in 1990s from *Pseudomonas* isolate from Japan and Italy respectively. Subsequently, these enzymes gradually expressed in a number of Enterobacteriaceaegenera. Both VIM and IMP now are endemic in Greece, Italy, Spain, Taiwan, and Japan, although outbreaks and increasing reports of the isolates have come from Europe. Cases with bacteria expresses IMP and VIM also noticed in many other countries, USA, and other South American countries. Besides, NDM-1 containing Enterobacteriaceaehad also been

reported in every continent of the world, direct link to the Indian subcontinent (India, Pakistan, and Bangladesh) was established most of these cases. It also reported in some African countries, like Morocco, Kenya, and South Africa(6, 13).

Class D includes OXA carbapenemases which are mostly found in *Acinetobacter spp*., although OXA-48 occurs in Enterobacteriaceae. High level of carbapenem resistant occurs only when OXA enzymes are co-expressed with ESBLs and porin resistance factors and mostly recovered from *K. pneumoniae* and *E.coli*, reported across Europe, the Southeastern Mediterranean region, and Africa(6, 14).

Emerging Resistance to carbapenems and their spread all over the world emphasizes the need to evaluate the burden of CPE. There is growing evidence of increasing prevalence of MDRE in different part of the world(15); however, as far as our concern of literature review information regarding the prevalence of CPE is not yet documentedand this is presumed to be first of its kind in Ethiopia.Therefore, this study was aimed to evaluate the prevalence, associated risk factors for CPE and MDREamong patients with UTI at the University of Gondar Hospital, Northwest Ethiopia.

2. LITERATURE REVIEW

2.1. Prevalence of multidrug resistant and carbapenemase producing Enterobacteriaceae

β-lactamase production is the most common mechanism of β-lactam drug resistance in gram negativebacteria. The rapid dissemination of MDRE has been increasingly reported and constitutes a major public health concernin both developing and developed world.For instance increased rate of MDRE were reported from American studies, Chicago(19.06%), *E.coli* the main MDR isolate (76%), followed by *Proteus mirabilis* and *Citrobacter* spp (6%), and *K. pneumoniae* (5%) (16). In Europe, similar finding from Italy and Belgium, the prevalence of MDRE were 62%; in both studies *K.pneumoniae* and *E.coli* were the mostprevailing MDR strains isolated(17, 18).

Multidrug resistant Enterobacteriaceaehave also a major public health threatin developing countries. According to study in Nepal in 2012 among 202 Enterobacteriaceae isolates, 40.1% were MDRE, *Citrobacter* spp were the principal MDR isolate (72%) followed by *E. coli* (38.2%)(19). However, in a 2013 another study from Nepal showed that increased prevalence of MDRE (64.04%)was reported. *E.coli* (74%) and *K.pneumoniae* (44%) were the predominant MDR isolates(20). Besides, in Mozambique, 88.2% of isolates of Enterobacteriaceaewere found to be MDR strains(21). A multicenter study in Senegal demonstrated that increased prevalence of antimicrobial resistance observed among Enterobacteriaceaeuropathogens. The overall resistance rates of ampicillin, amoxicillin, amoxicillin-calvulanic acid, naldixic acid, fluoroquinolones and cotrimoxazole were77.3%, 34.7%, 14.7%, 13.3% and 55%, respectively. Among member of Enterobacteriaceae, 89% of drug resistances were implicated in *E. coli* and *K. pneumoniae*(22).

Various research findings claimed that increased trendsin the prevalence of MDR among Enterobacteriaceae also a major concernin Ethiopia. Particularly in Gondar the prevalence of MDRE increased from time to time, i.e. 2002 the prevalence of MDR strains were 68%(23), the magnitude increased to 85.5% (2007)(24) and 93.5% (2013)(25). Moreover; tremendous prevalence of MDR strains also reported in other parts of Ethiopia, in Dessie (74.6%) (26), Bahirdar (95.6%) (27), and Jimma (100%) (28). In all studies *E. coli* and *K.pneumoniae* were the principal MDR isolates among uropathogens.

In the recent years, there is an increase trend of resistance to carbapenem among Enterobacteriaceae in clinical isolates. According to data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) showed that, the rates of CPE (*K. pneumoniae*) increased in most European countries. Greece, Cyprus and Italy reported resistance rates of 43.5%, 17.0% and 1.3% respectively (29-31). Likewise, based on center for disease control and prevention report on health care associated infections the overall prevalence of CPE (KPC) rising from less than 1% in 2000 to 8% in 2007 (15). Particularly, in France 6, 26 and 13 CPE episodes were reported in 2009, 2010 and the first four months of 2011, respectively (32).

A summary data from EARS-Net surveillance study (2011) starting from 2008, the numbers of confirmed CPE increased dramatically: 23 in 2008, 73 in 2009, 333 in 2010, and 561 in 2011. Most producers were *Klebsiella* (80%), mainly *K. pneumoniae*, followed by *E. coli* (10%) and *Enterobacter* (8%), with the remaining 2% comprising occasional isolates of *Citrobacter, Morganella, Providencia, Raoultella and Serratia*. The enzymes produced included KPC (62%), NDM (14%), VIM (12%), and OXA-48-like (9%) and IMP (2%) types(33).

Carbapenemase producing Enterobacteriaceae are the principal clinical isolates from patients with UTI. A four year study from Ontario, Canada; demonstrated that from known 73 CPE clinical isolates, 46.6%, 32.9%, 5.5%, 2.75%, 2.75%, 2.75%, 1.4%, 1.4%, & 4.1% were recovered from urine, rectal swab, wound, blood, sputum, intraperitoneal fluid, bone, skin swab & other body samples respectively (34). Besides, similar isolation rate of carbapenemase producing *K. pneumoniae* detected from patients with UTI in Italy and it was 47.5% (35).

According to a study, from 4564 screened Enterobacteriaceae isolates, 158 (3.5%) were carbapenemase producing strains. *K. pneumoniae, E.coli, K. oxytoca* and *Enterobacter* spp had found expressing different class of carbapenemase (36). Moreover, comprehensive epidemiological study in United Kingdom, India and Pakistan showed that about 4.5% of isolates were CPE (37). In another study the overall prevalence of CPE had been found 10.9%, *K. pneumoniae* (90%) and *E.coli* (10%) were most important isolates (38). A finding from United States of America, the prevalence of CPEwas found to be 21% (39).

In Asia, increase prevalence of CPE was also reported, in India Among 392 isolates of Enterobacteriaceae and gram negative non-fermentative bacilli, carbapenemase production was detected in 21 (5.4%) isolates (40).Besides, anotherfinding from India showed that 12.9% of isolates were CPE (41). Finding from Pakistan, the presence of CPE was investigated using chromogenic culture media, 13isolates (8·6%) were found to be NDM-1 producing Enterobacteriaceae (42). In Bangladesh, among the isolates, 4.8% were found to be CPE (43). Increase prevalence of CPE has been also reported from 64 isolated strains, 13 (20.3%)mainly *K. pneumoniae*(44), in the same year, another study in Iran relatively low prevalence of CPE were also documented (14.7%) (45). While, a study in Taiwan has shown that decreased incidence of CPE (2.5%) was reported compare to other studies in this continent (46).

Due to lack of sufficient surveillance study in Africa, only few studies are reported. In Kano, Nigeria the prevalence of CPE were reported to be 14%. High carbapenemase expression detected in *K. pneumoniae* followed by *Proteus* spp &*E.coli* (47). Another finding from Nigeria, extremely high prevalence of CPE, reported i.e. 33.5% (67/200) isolates were found to be carbapenemase producing strains; of which, *E. coli* accounts(31.3%), followed by *Proteus* spp (21.6%) and *K. pneumoniae* (14.3%) (48). A study in Morocco has shown the overall prevalence of CPE were 13/463 (2.8%). These were *K. pneumoniae* (69.2%), *E .coli*(23%) and *K. oxytoca* (7.8 %), showed 100% resistant to AMC, CTX, CTR, and SXT; 85%resistant to CIP and 54%susceptible to GEN(49). From November 2008 through October 2009, 11 CPE isolates (8 *Klebsiella pneumoniae*, 1 *Escherichia coli*, 1 *Enterobacter cloacae*, and 1 *Enterobacter sakazakii*) were identified at the Institute Pasteur (Dakar, Senegal) (50). Furthermore, a study in Kenya from archival collected MDR *K. pneumoniae* strains, seven carbapenem resistant carbapenemase (NDM-1) were isolated from urine, the isolates were resistant to all b-lactam drugs including carbapenems(51).

2.2. Risk factors of multidrug resistant and carbapenemase producing Enterobacteriaceae

Several factors are associated with the rapid increase in the prevalence of MDRE. Based on a retrospective study, age, gender, diabetes mellitus, obstructive uropathy, health care associated risks (chronic indwelling urinary catheters, healthcare exposure, including hospital stay for at least 48 hours, nursing home or long-term carefacility, residence, regular hemodialysis clinic

visits or urological procedures within the past 3 months), prior UTI, and prior use of any antibiotics, were associated with MDRE infections(16).

Rapid spread and increase prevalence of CPE attributed by various factors. According to a systematic review by experts from European center for disease prevention and control(ECDC); risk factors found to be associated with colonization or infection with CPE were: advanced age, prior antimicrobial use, length of stay (time at risk); severity of illness; mechanical ventilation; admission to the intensive care unit (ICU), high procedure score; presence of wounds, transfer between hospital units within the same hospital, chronic disease(acquired immuno-deficiency syndrome, chronic heart disease, chronic lung disease); diabetes mellitus; pregnancy; prior surgery; prior hospital stay; presence of a biliary/urinary catheter, previous UTI and recent transplantation(16, 52, 53).

Furthermore, several investigators have also evaluated the factors associated with increased risk for acquisition of CPE. A retrospective case control study has shown that the independent risk factors for infection with CPE were prior fluoroquinolones use, previous receipt of a carbapenem drug, admission to the ICU, and history of antibiotic use(54). Similarly finding from Israel, independent predictors of subsequent CPE clinical specimens were: admission to the ICU, hospital stay, having urinary catheter, receipt of antibiotics, and diabetes mellitus(55).

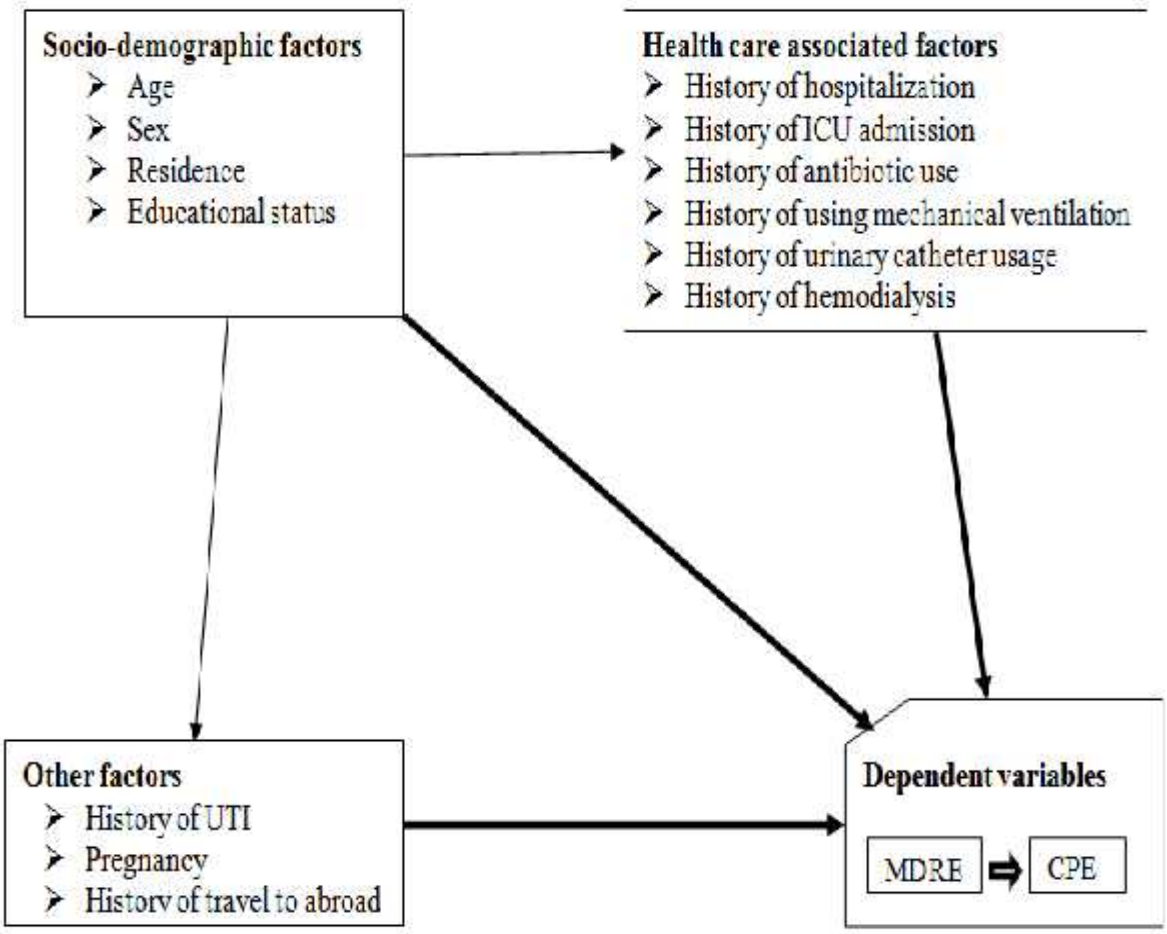


Figure 1: Conceptual framework for factors associated with MDRE and CPE.

3. SIGNIFICANCE OF THE STUDY

The aetiology of UTI and the antibiotic resistance of uropathogens have been changing over the past years, both in community and health care associated infections. Current knowledge on the burden and antimicrobial susceptibility pattern of the Enterobacteriaceae isolatesis essential for appropriate therapy, since those groups of bacteria are the main cause of UTIs and possess several mechanisms to dismantle currently available antibiotics including carbapenems.

This study is important to provide baseline and crucial information regarding to MDR and CPE isolates among UTI patients. Hence policy makers, health administrators and other stake holders can be benefited to design and implement appropriate intervention mechanisms to combat the incidence of infections caused by resistant strains in particular. Furthermore, asa result of this finding health care providers will enforce to follow empirical treatment rules, adopt and utilize standard protocols in the identification of CPE as routine activities.

Moreover, there are few studies conducted in the continent of Africa, based on the knowledge of literature review no published findings were found on the aetiology and resistance pattern of community and hospital acquired UTIs caused by CPE in Ethiopia. Therefore, the purpose of this study was to determine the prevalence, associated risk factors of carbapenemase producing and MDRE among patients with symptomatic UTIs at the University of GondarHospital, Northwest Ethiopia.

4. OBJECTIVES

4.1. General objective

- To assessthe prevalence and risk factors ofmultidrug resistant andcarbapenemase producing Enterobacteriaceaeamong patients with symptomatic urinary tract infection, University of Gondar Hospital.

4.2. Specific objectives

- To determine the prevalence of multidrug resistant Enterobacteriaceae
- To determine the prevalence of carbapenemase producing Enterobacteriaceae
- To identify risk factors relating with the occurrence of multidrug resistant Enterobacteriaceae
- To identify risk factors relating with the occurrence of carbapenemase producing Enterobacteriaceae

5. MATERIALS AND METHODS

5.1. Study area

The study was conducted at the University of Gondar Hospital. The University of Gondar Hospital is a tertiary level teaching hospital which is located in Gondar town, 750 km from Addis Ababa in the Northwest Ethiopia. The hospital provides surgical, medical, pediatric, gynecologic, obstetric, and ophthalmologic services to the community for over 5million inhabitants. The hospital has an accredited (three star) referrallevel laboratory with 7 sections and a separate reception room. Microbiology section is one of the principal area, it is estimated that 9,600 samples delivered per annum to this working area. In this section, culturing is one of the main activities, mainly applicable for bacterial isolation and identification.

5.2. Study design and period

A laboratory based cross-sectional study was conducted from Februaryto May 2014.

5.3. Population

5.3.1. Source population

Source of population was all patients with suspected UTI seeking treatment, at the University of Gondar Hospital.

5.3.2. Study population

Patients with symptomatic suspected UTIs, whowere accessed at the time of study period at theUniversity of Gondar Hospital.

5.4. Inclusion criteria

Patients with symptomatic UTIwere involved in the study

5.5. Exclusion criteria

Patients with asymptomatic UTI and patient on antibiotics (since the last 7 days)were excluded from the study.

5.6. Study variables

5.6.1. Independent variables

Age, sex, history of travel to abroad, prior antibiotic use in the past 6 months, history of UTI in the past 12 months, history of hospitalization in the past 12 months, ICU admission in the last 6 months, surgery in the last 6 months, history of urinary catheter usage in the past 12 months, presence of mechanical ventilator in the past 12 months, pregnancy and history of hemodialysis in the past 3 months.

5.6.2. Dependant variables:

- Presence/Absence of MDRE
- Presence/Absence of CPE

5.7. Sampling technique and Sample size determination

Systematic random sampling technique was utilized. Sample size was determined by using the prevalence of 50%. So that the final sample size calculated by using singlepopulation proportion formula.

$$N = \frac{Z(\alpha/2)^2 xp(1-p)}{d^2}$$, where

- N is the minimum sample size
- $Z_{\alpha/2}$ is the standard normal deviation corresponding the specified of total Population, at 95% confidence level = 1.96
- p is the prevalence = 0.5; 1-p = 0.5, and d is the desired degree of accuracy = 0.05

So that a total of 442 study participants with symptomatic UTIwere enrolled, considering 15% contingency.

5.8. Sampling procedure

A total of 442 study participants were selected by using systematic sampling interval (K), calculated by using the anticipated patient (UTI) flow in hospital; approximately 420 patients/month. So that data collection planned for 3 months: K= N/n, 1260/442 = 3. Then, the first three individuals were selected;of whom one individual was included in the study randomly. Finally, every 3rd individual who had visited the hospitalwas selected to participate in the study.

5.9. Operational definition

Hospital acquired infection: infection acquired during hospital stay and that appears within 48-72 hours after admission in the hospital and the patient was not incubating this infection at the time of admission.

Multidrug resistant(MDR): defined as resistance to two or more different antibioticagents.

5.10. Data collectionand processing

5.10.1. Questionnaire

Important variables of the study were addressed by using interviewer administered structured questionnaire (mainly adopted from ECDC survey, 2013) having three parts; the first contains socio-demographic information, the second part comprises clinical data and the 3rd part contains possible risk factors for CPE and MDRE**(Annex-I).**The questionnairewas translated to Amharic and re-translated back to English to make the reliability of the instrument. Before undertaking the data collection the instrument was tested taking 5% eligible for the feasibility of the questionnaires. Data were collected by two trained data collectors, experiencednurse/health officerand medical laboratory profession, previous experience and living and speaking local language. Data were gathered mainly through patient interview and revising medical records.

5.10.2. Urine specimen

Urine specimen was collected from each patient, after instructing how to collect a clean catch mid-stream urine specimen(**Annex-II)**(56). Accordingly, about 20 ml urine specimen was collected in a sterile screw-capped, wide-mouth container and labeled with the unique sample number, date and time of collection. Urine from infants and severely ill patients were collected by health professionals.The specimens were delivered to bacteriology laboratory within 30 minutes of collection for culture and microscopic examinations. The investigator and a trained laboratory technician/ technologist had undertaken activities in each stage of urinalysis (pre-analytical, analytical and post- analytical) to gather the intended laboratory information.

5.10.3. Isolation and Identification of Enterobacteriaceae

Urine specimens obtained from the patients weredirectly inoculated on 5% Sheep blood agar (**Annex-III**). Culture plates wereincubated at 37ºC for 24 hours, after incubation urine culture is considered as positive, if it contains $>10^5$ cfu/ml of clean catch mid-stream urine. After gram staining pure colonies (convex, grey, smooth/mucoid)were sub-cultured on MacConkey agar

(**Annex-III**) for further identification. Enterobacteriaceae from positive urine cultures were identified by their characteristic appearance on the media, gram staining reaction, by the pattern of biochemical profiles using standard procedures.Biochemical tests such as indole production, sugar fermentation, H_2S and gas production, citrate utilization, motility test, urease test, oxidase, were used to identify Enterobacteriaceae isolates (56).

5.10.4. Antimicrobial susceptibility testing

All identified clinical strains were subjected to in vitrosusceptibility testing using Kirby Bauer disk diffusion method as described in clinical and laboratory standards institute (CLSI) guidelines and interpreted accordingly (57).From a pure culture 3-5 selected colonies of bacteria were taken and transferred to a tube containing 5 ml sterile nutrient broth (Oxoid) and mixed gently until a homogenous suspension was formed and incubated at 37^oC until the turbidity of the suspension become adjusted to a McFarland 0.5. A sterile cotton swab was used and the excess suspension was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller Hinton agar (**Annex-III**) (pH 7.2-7.4) (Oxoid) (57).

After inoculation, the following antibiotic disks (Oxoid)wereequidistantly placed on these plates and gently pressed onto themedium with the help of sterile forceps to ensure complete contact with the agar surface.:cefotaxime (CTX; 30µg),ceftriaxone (CTR; 30µg), cefepime (CPM; 30µg), ceftazidime (CAZ; 30µg), cefpodoxime (CPD: 30µg), ciprofloxacin (CIP; 5µg), tetracycline (TE; 30µg), chloramphenicol (C; 30µg), amoxicillin-calvulanic acid (AMC; 30µg), naldixic acid (NA; 30µg),gentamycin (GEN; 10µg), ampicillin (AMP; 10µg)and trimethoprim-sulfamethoxazole (SXT; 25µg). The criteria used to select the antimicrobial agents are based on their availability and frequent prescriptions for the management of UTIs. The plates were then incubated at 37oC for 24 hours. Diameters of the zone of inhibition around the discs was measuredusing antibiotic zone scale (HiMedia), and the isolates were classified as susceptible, intermediate and resistant(57).

5.10.5. Carbapenemase testing

After antimicrobial susceptibility testing, each bacterial isolate considered as MDRE was sub-cultured on CHROMagar^{TM}KPC agar**(Annex-III)**to determine carbapenemase production. After overnight incubation (18-24hr), carbapenemase producing isolates were assessed by visualizing colonies with typical coloring characteristics. Carbapenemase producing *E. coli*developed dark pink to reddish colony features, while other Enterobacteriaceae isolate produced metallic blue colonies(58).

5.10.6. Quality control

The reliability of the study findings were guaranteed by implementing Quality control measures throughout the whole process of the laboratory work. All materials, equipment and procedures were adequately controlled. Culture media were tested for sterility and performance. Pre-analytical, analytical and post-analytical stages of quality assurance that are incorporated in standard operating procedures of the microbiology laboratory of University of Gondar hospital were strictly followed. International control strains**;** *E.coli*®ATCC 25922(positive control) and *S. aureus* ATCC®25923 (negative control)were used to control the performance of media. To standardize the inoculum density of bacterial suspension for a susceptibility test, 0.5 McFarland standardswas used(57, 58).

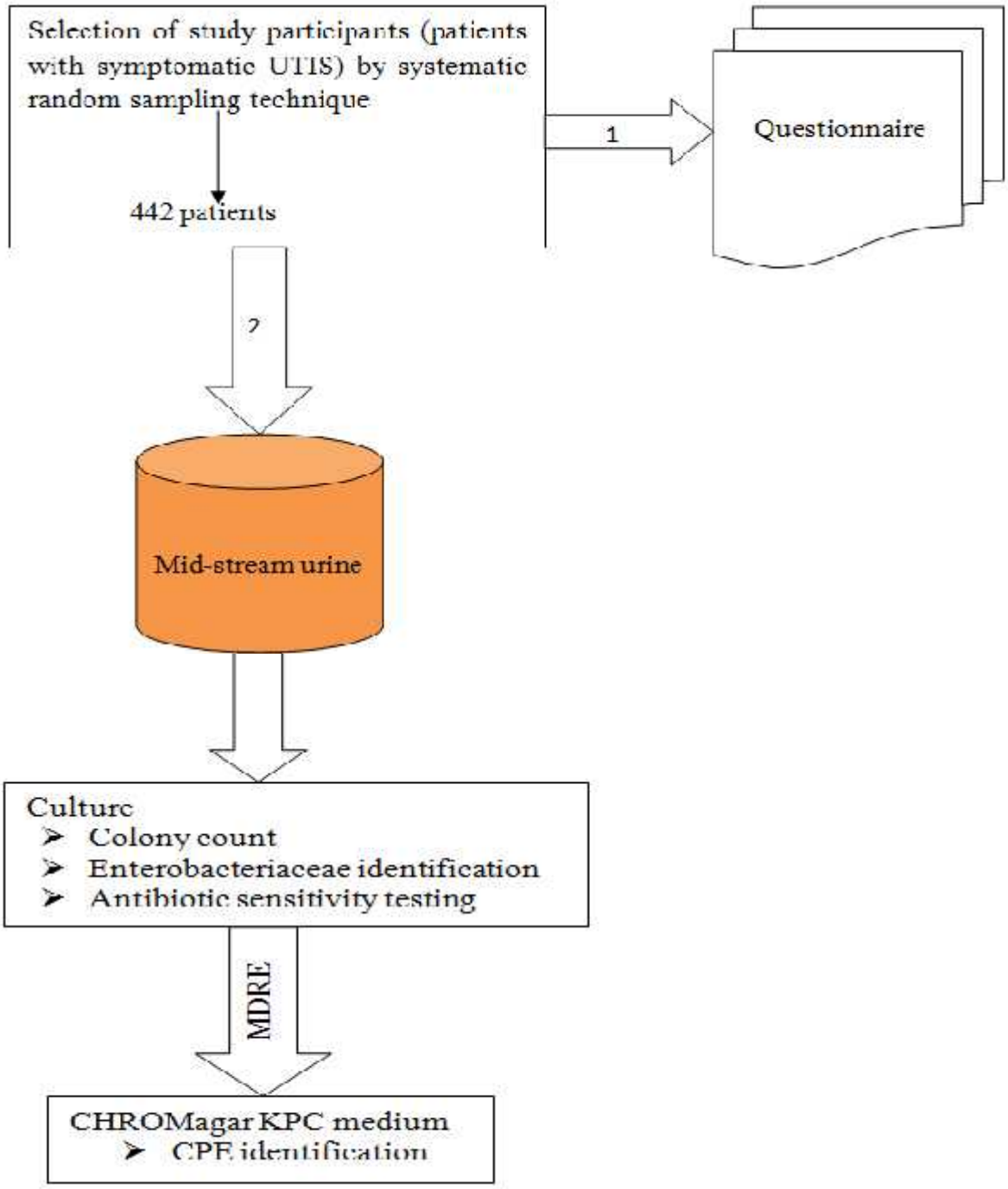


Figure 2: Flow chart explaining the experimental work.

5.11. Data Analysisand interpretation

Data were collected, summarized, tabulated and analyzed using SPSS version 20 software and resultswere presented through tables, pie charts and graphs. Associationswere measured using chi-square test, binary logistic regression. P-values< 0.05 were considered as statistically significant.

5.12. Ethical Considerations

The study was initiated after ethical approval by Research and Ethics committee of School of Biomedical Laboratory Sciences. Subjects were recruited after getting written informed consent. Only those who are volunteerswererequested to give samplesand to answer intended questions. Participantshad full right to continue or withdraw from the study. For each confirmed case, the responsible clinician of the patient was informed and gets their treatment timely. Information obtained in each course of the study was kept confidential.

5.13. Result dissemination

The result will be disseminated to University of Gondar, College of Medicine and Health Sciences, School of Biomedical and Laboratory Sciences**,** Department of Medical Microbiology and other concerned bodies. In addition, the study will be presented to the health staffs in health facility where the study is conducted. Moreover the finding of the study will be also presented to GCMHS staffs and students. Finally a manuscript will be prepared and submitted for publication in areputable journal.

6. RESULTS

6.1. Socio-demographic characteristics

A total of 442 patients with symptomatic UTI were included in this study to investigate prevalence and risk factors of MDRE and CPE. The majority of the participants were females 282 (63.8 %). Themean age of patients was 37.05±10.5 years, 86 (19.5%) of the patients were younger than 16 years, and 73 (16.5%) were older than 60 years. Two hundred fifty two (57.0%) of patients were residents of rural areas, and majority, 286 (64.7%) of study participants had educational level of elementary school and below (Table 1).

Table 1: Socio-demographic characteristics of study participants: University of Gondar Hospital, February to May 2014 (N = 442).

Variables		Frequency	Percentage
Sex	Male	160	36.2
	Female	282	63.8
Age	≤15	86	19.5
	16-30	84	19
	31-45	101	22.9
	46-60	98	22.2
	≥61	73	16.3
Residence	Rural	252	57
	Urban	190	43
Educational status	Illiterate	196	44.3
	Primary school	90	20.4
	Secondary school	69	15.6
	Diploma and Above	87	19.7
Sender of the patient	Outpatient	212	48
	Inpatient	230	52

6.2. Prevalence of MDRE and CPE isolates among study participants

Among study participants, 183 (41.4%) patients, who had positive urine culture with a single non-duplicate isolates of Enterobacteriaceaewere identified. The majority of isolates were *E.coli* 112 (61.2%) followed by *K. pneumoniae*29 (15.8%) and *E.aerogenes* 13(7.1%) (Figure3). The isolates were tested for antimicrobial susceptibility, 160 (87.4%, 95%CI; 82-92.3%)of themshowed resistance to two or more antibiotics. Among MDR strains, only 1 (0.6%) isolate was resistant to 2 antibiotics, the rest 159 (99.4%) were resistant to three or more antibiotics (Table 2). From MDRE isolates, 106 (66.3%) were identified from female patients and 37 (23.1%) of isolates were indicated in patients with age group from 31 – 45 years (Table3).

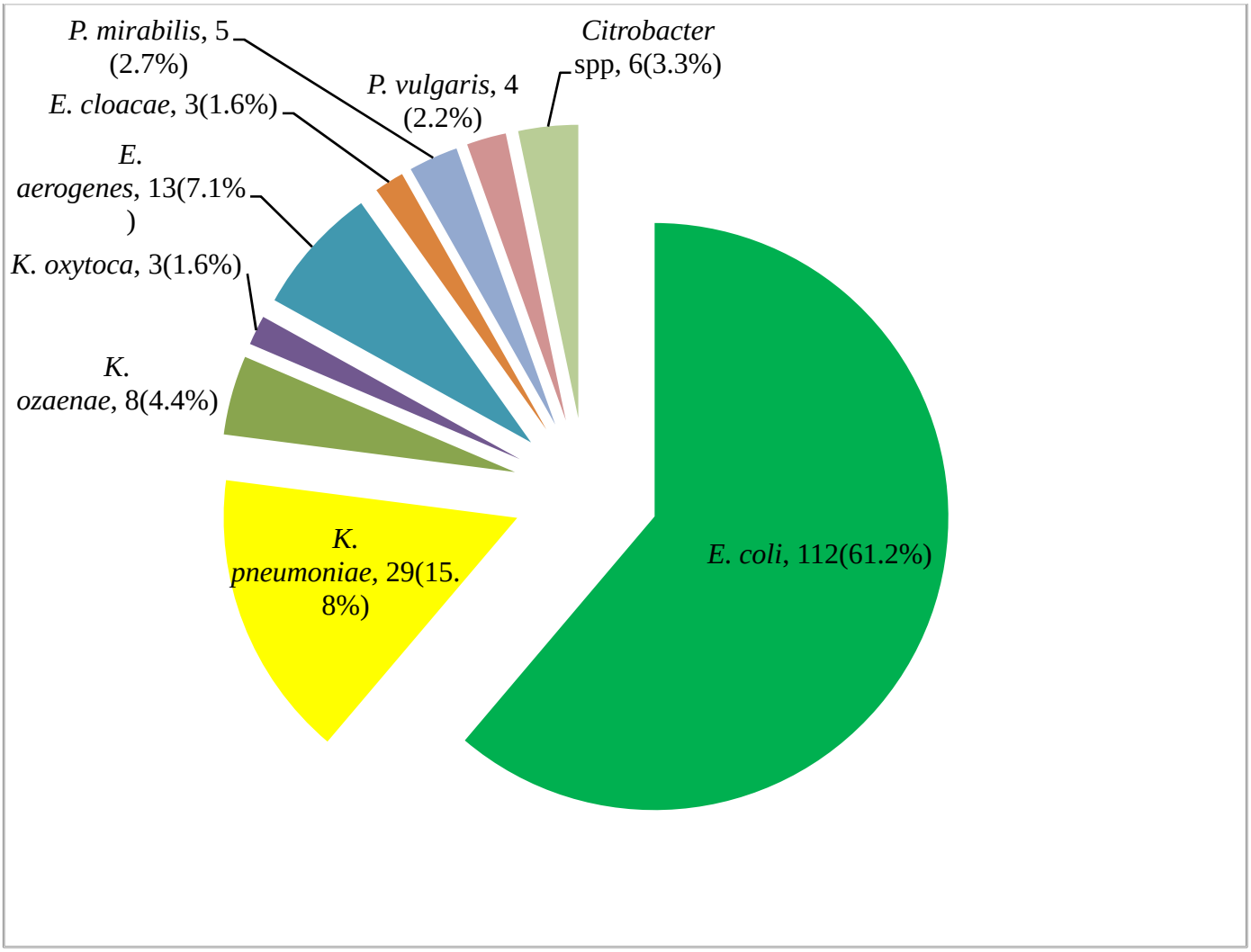


Figure 3: Frequency of Enterobacteriaceae isolatesamong study participants: University of Gondar Hospital, February to May 2014.

Table 2: Multidrug resistance pattern of Enterobacteriaceae among study participants: University ofGondar Hospital, February to May 2014.

Isolates	Degree of resistance										MDR isolates
	R0	R1	R2	R3	R4	R5	R6	R7	R8	≥R9	(≥R2)
E. coli(N = 112)	2 (1.8)	6 (5.4)	—	5 (4.5)	28 (25)	24 (21.4)	32 (28.6)	9 (8.0)	4 (3.6)	2 (1.8)	104 (92.9)
K. pneumoniae(N = 29)	—	1 (3.4)	—	3 (10.3)	5 (17.2)	6 (20.7)	4 (13.8)	3 (10.3)	4 (13.8)	3 (10.3)	28 (95.6)
Other *Klebsiella* spp.(N = 11)	—	2 (18.2)	—	—	—	3 (27.3)	3 (27.3)	1 (9.1)	1 (9.1)	1 (9.1)	9 (81.8)
Enterobacter spp. (N = 16)	2 (12.5)	1 (6.3)	—	1 (6.3)	2 (12.5)	3 (18.8)	5 (31.3)	1 (6.3)	—	1 (6.3)	13 (81.3)
Citrobacter spp. (N = 6)	1 (16.7)	—	1 (16.7)	—	1 (16.7)	—	2 (33.3)	—	1 (16.7)	—	5 (83.3)
Proteus spp. (N = 9)	5 (55.6)	3 (33.3)	—	—	—	1 (11.1)	—	—	—	—	1 (11.1)
Total (N = 183)	10 (5.5)	13 (7.1)	1 (0.5)	9 (4.9)	36 (19.7)	37 (20.2)	46 (25.1)	14 (7.7)	10 (5.5)	2 (1.1)	160 (87.4)

Note: Data are in number (%) unless otherwise indicated.
R0: susceptible to all antibiotics, R1-8: resistance to 2, 3, 4, 5, 6, 7, and 8antibiotics, ≥R9: resistance to 9 or more antibiotics, ≥R2: resistance to 2 or more antibiotics.

Table 3: Distribution of MDRE and CPEper socio-demographic characteristics among study participants: University of Gondar Hospital, February to May 2014.

Characteristics		MDRE isolates		p-value	CPE isolates		p- value
		Yes	No		Yes	No	
Sex	Female	106	12	0.187	4	102	0.509
	Male	54	11		1	53	
Age group	≤15	27	16	**<0.001**	1	26	0.277
	16 - 30	31	4		0	31	
	31 – 45	37	2		3	34	
	46 – 60	33	1		1	32	
	≥61	32	0		0	32	
Residence	Rural	85	16	0.138	0	85	**0.016**
	Urban	75	7		5	70	
Educational status	illiterate	70	14	0.331	2	68	0.510
	Primary school	32	5		1	31	
	Secondary school	29	2		2	27	
	Diploma and above	29	2		0	29	
Sender of patient	Outpatient	67	13	0.185	1	66	0.314
	In patient	93	10		4	89	

The distribution of the bacteria in patients' with and without MDRE isolates is shown in Table 4. *E.coli* was significantly more common in patients with MDRE than those with non-MDRE infections (65 versus 34.8%; $P = 0.020$), while *P. vulgaris*(0.63% versus 13%; P = 0.000) and *E.cloacae* (0.63% versus 8.7%; p = 0.004) were significantly more common in patients with non-MDRE than those with MDRE infections. Moreover; *K. oxytoca* and *P. mirabilis* were only identified in patients with MDRE and non-MDRE infections, respectively.

Table 4: Enterobacteriaceae isolates among patients with MDRE and non-MDRE UTIs: University of Gondar Hospital, February to May 2014.

Isolates	MDRE isolates (N = 160)	Non-MDRE isolates (N = 23)	P- value
E.coli	104 (65%)	8 (34.8%)	**0.020**
K.pneumoniae	28 (17.5%)	1 (4.3%)	0.106
K.ozaenae	6 (3.8%)	2 (8.7%)	0.282
K.oxytoca	3 (1.9%)	0 (0.0)	___
E.aerogenes	12 (7.5%)	1 (4.3)	0.582
E.cloacae	1 (0.63%)	2 (8.7%)	**0.004**
P.mirabilis	0 (0.0)	5 (21.7%)	___
P.vulgaris	1 (0.63%)	3 (13%)	**<0.001**
Citrobacter spp.	5 (3.13%)	1 (4.3%)	0.758

Of the 183 Enterobacteriaceaeisolates, 160 (87.4%) were MDR strains, and these strains were tested for carbapenemase production by using phenotypic methods (CHROMagar KPC media). A total of 5 bacterial strains were found to be CPE, Notably *E.coli* (2), *K. pneumoniae* (2) and *E. aerogenes* (1) (Figure 4). The overall prevalence of CPE was 2.73% (95%CI; 0.5-5.5%) among all isolates and 3.1% among MDRE isolates, 4/5 (80%) of CPE isolates were identified from females and 3/5 (60%) isolates were also indicated from patients with age group from 31 – 45 years (Table 3). Besides, all CPE strains were 100% ESBL producer, which were demonstrated by using phenotypic methods (CHROMagar ESBL media).

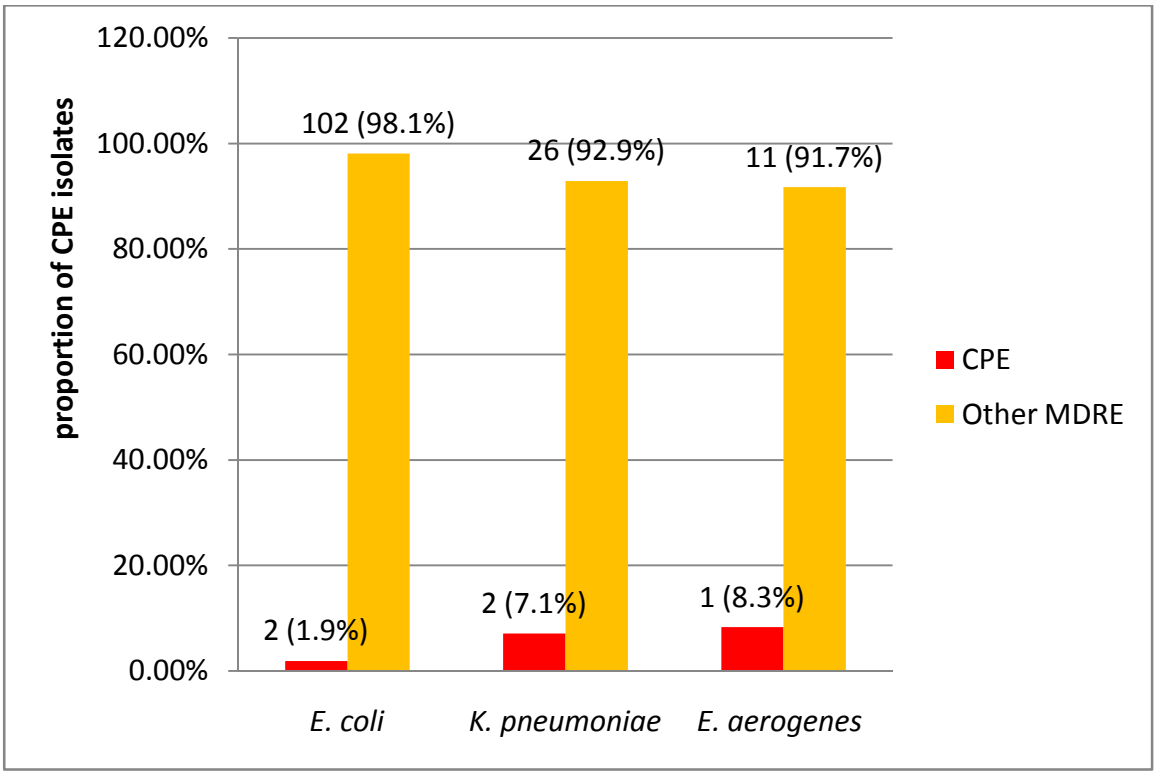


Figure 4: Proportion of CPE from MDRE isolates among study participants: University of Gondar Hospital, February to May 2014.

6.3. Risk factors for MDRE and CPE among study participants

Risk factors associated with MDRE UTIs were analyzed by comparing patients with and without MDRE UTIs. By bivariate analysis (Table 5), age, hospitalization for the last 12 months, prior urinary tract infection for the past 12 months, prior antibiotic use for the past 6 months were associated with MDRE infections. In the multivariate logistic regression analysis (Table 5), independent risk factors for MDRE were prior antibiotic use, and hospitalization since the past 12 months, age, and sex (female).

Risk factors for CPE among patients with UTIs were analyzed by comparing patients with and without CPE. However, none of the investigated factors (age, hospitalization for the last 12 months, prior urinary tract infection, and prior antibiotic use) were found as risk factor for CPE among patients with UTIs.

Table 5: Risk factors associated with MDRE among study participants: University of Gondar Hospital, February to May 2014.

Risk factors	MDRE		Bivariate analysis		Multivariable analysis	
	Yes (N = 160)	No (N = 23)	COR (95% CI)	P-value	AOR (95% CI)	P-value
Sex						
Female	106	12	1.79 (0.75 – 4.34)	0.191	4.46 (1.29 – 15.35)	**0.018**
Male	54	11	1		1	
Age (years)						
Mean age	38.5	13.2	1.08 (1.05 - 1.12)	< **0.001**	1.08 (1.03 – 1.13)	**0.001**
Hospitalization						
Yes	114	9	3.86 (1.56 – 9.53)	**0.003**	5.22 (1.59 – 17.17)	**0.006**
No	46	14	1		1	
Prior UTI						
Yes	65	4	3.25 (1.06 – 9.99)	**0.040**	2.41 (0.56 – 10.34)	0.239
No	95	19	1		1	
Prior antibiotic use						
Yes	129	7	9.51 (3.60 – 25.11)	< **0.001**	3.98 (1.056 – 14.97)	**0.041**
No	31	16	1		1	

Note that: COR: crude odds ratio, AOR: adjusted odds ratio, CI: confidence interval

6.4. Antibiotic resistance pattern of MDRE and CPE among study participants

The overall resistance profile of MDRE isolates are shown in Table 6. High resistance rate were observed to ampicillin (97.5%) followed by cotrimoxazole (64.4%), and chloramphenicol (61.2%). Whereas, ciprofloxacin, cefepime, and ceftriaxone had an overall resistance rates of 2.5%, 10.6%, and 11.9%, respectively. Species specific antibiotic resistance rates are also presented in Table 6. *E.coli,* the most frequently isolated bacterium, showed that more than 55% of strains were resistant to ceftazidime, gentamycin, chloramphenicol, cotrimoxazole, and ampicillin and low rates of resistance rates were noted to ciprofloxacin (1%), cefepime (8.7%) and ceftriaxone (11.5%). *K. pneumoniae*the second most common isolate exhibited over 60% of strains were resistance to amoxicillin-calvulanic acid, chloramphenicol, cefpodoxime, and ampicillin, relatively low resistance rates were indicated to ciprofloxacin (10.7%), cefepime (14.3%), and ceftriaxone (17.9%).

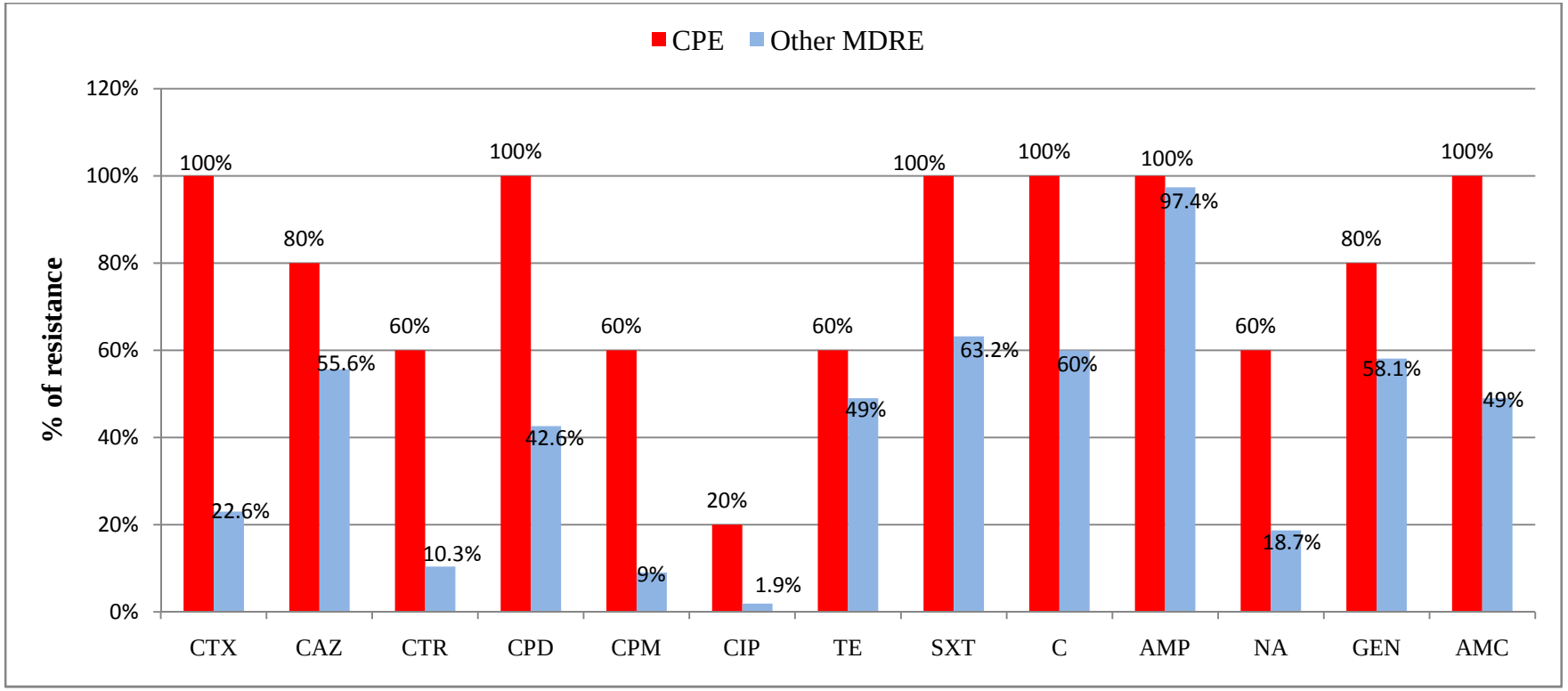
Additionally, the overall resistance pattern of CPE isolates are summarized in Figure 5. All isolates were 100% resistant to cefotaxime, cefpodoxime, cotrimoxazole, chloramphenicol, ampicillin, and amoxicillin-calvulanic acid. However, only 20% of strainswere resistant to ciprofloxacin. Moreover, as shown from Figure 5, the overall antibiotic resistance rates of CPE isolates were significantly higher than other MDRE strains for more than half of tested antibiotics including cefotaxime (100% versus 22.6%; $P<0.001$), ceftriaxone (60% versus 10.3%; $P=0.001$), cefpodoxime (100% versus 42.6%; $P = 0.011$). On the other hand the difference in antibiotic resistance rate of CPE to ceftazidime, tetracycline, cotrimoxazole, chloramphenicol, ampicillin, and gentamycin were not statistically significant compared to other MDRE isolates.

Table 6: Antibiotic resistance patterns of MDRE among study participants: University of Gondar Hospital, February to May 2014.

MDR isolates	Antibiotics												
	CTX	CAZ	CTR	CPD	CPM	CIP	TE	SXT	C	AMP	NA	GEN	AMC
E.coli(N=104)	25 (23.1)	58 (55.8)	12 (11.5)	43 (41.3)	9 (8.7)	1 (1)	49 (47.1)	72 (69.2)	61 (58.7)	103 (99)	19 (18.3)	59 (56.7)	47 (45.2)
K.pneumoniae(N = 28)	8 (28.6)	16 (57.1)	5 (17.9)	18 (64.3)	4 (14.3)	3 (10.7)	15 (53.6)	14 (50)	18 (64.3)	26 (92.9)	7 (25)	16 (57.1)	17 (60.7)
K. ozaenae (N = 6)	1 (16.7)	4 (66.7)	0	3 (50)	0	0	4 (66.7)	5 (83.3)	5 (83.3)	6 (100)	2 (33.3)	4 (66.7)	5 (83.3)
K. oxytoca (N = 3)	1 (33.3)	2 (66.7)	0	1 (33.3)	1 (33.3)	0	1 (33.3)	2 (66.7)	2 (66.7)	3 (100)	1 (33.3)	3 (100)	1 (33.3)
E. aerogenes (N = 12)	4 (33.3)	7 (58.3)	2 (16.7)	5 (41.7)	3 (25)	0	5 (41.7)	5 (41.7)	7 (58.3)	12 (100)	2 (16.7)	7 (58.3)	9 (75)
E. cloacae (N = 1)	1 (100)	1 (100)	0	0	0	0	0	1 (100)	0	1 (100)	0	0	0
Citrobacter spp (N = 5)	1 (20)	2 (40)	0	1 (20)	0	0	4 (80)	3 (60)	4 (80)	4 (80)	1 (20)	4 (80)	2 (40)
P. vulgaris (N = 1)	0	0	0	0	0	0	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	0
Total (N = 160)	40 (25)	90 (56.2)	19 (11.9)	71 (44.4)	17 (10.6)	4 (2.5)	79 (49.4)	103 (64.4)	98 (61.2)	156 (97.5)	32 (20)	94 (58.8)	81 (50.6)

Note: Data are in number (%) unless otherwise indicated.

CTX: Cefotaxime, CAZ: Ceftazidime, CTR: Ceftriaxone, CPD: Cefpodoxime, CPM: Cefepime, CIP: Ciprofloxacin, TE: Tetracycline, SXT: Cotrimoxazole, C: Chloramphenicol,NA: Naldixic acid, GEN: Gentamycin, AMC: Amoxicillin-Calvulanic acid.



	Antibiotics												
	CTX	CAZ	CTR	CPD	CPM	CIP	TE	SXT	C	AMP	NA	GEN	AMC
P-value[a]	**<0.001**	0.277	**0.001**	**0.011**	**<0.001**	**0.011**	0.629	0.091	0.071	0.716	**0.023**	0.327	**0.025**

Note that: a: compared between CPE and other MDRE
CTX: Cefotaxime, CAZ: Ceftazidime, CTR: Ceftriaxone, CPD: Cefpodoxime, CPM: Cefepime, CIP: Ciprofloxacin, TE: Tetracycline, SXT: Cotrimoxazole, C: Chloramphenicol,NA: Naldixic acid, GEN: Gentamycin, AMC: Amoxicillin-Calvulanic acid.

Figure 5: Antibiotic resistance rate of CPE isolates compared to other MDRE among study participants: University of Gondar Hospital, February to May 2014.

7. DISCUSSION

The present study showed that the prevalence of MDR among Enterobacteriaceae isolates identified from patients with symptomatic UTI was 87.4% (95% CI; 82-92.3%), which is slightly similar with the results reported in Gondar (85.5%) and Mozambique (88.2%) (21, 24). While, relatively low prevalence of MDR uropathogens weredemonstrated in Ethiopia;Gondar (68%), and Dessie (74.6%) (23, 26). Moreover, the present result is also much higher than reports from other countries, such as Chicago (19.06%), Belgium (62%), and Italy (62%), Nepal (40.1%, 64.04%) (16-20). However, it is lower than reports in other studies from Gondar (93.5%), Bahirdar (95.6%), and Jimma (100%) (25, 27, 28). We postulate that, variation in prevalence of MDRE could be due to increase trend of MDR strains with time, and difference in geographical location, study period, study population, study design, method employed for each study.

E.coli (65%) and *K. pneumoniae* (15.6%) are the principal MDR isolates in this study, which are comparable with other previous studies conducted in different part of Ethiopia, i.e. Gondar, Dessie, Bahirdar and Jimma (25–28). Likewise the finding from Nepal, *E.coli* (74%) and *K.pneumoniae* (44%) were also the predominant MDR uropathogens (20), the same situation also demonstrated in Senegal, Chicago, Italy, and Belgium (16–18, 22). However, another study in Nepal showed that *Citrobacte*r spp. (72%) were the main MDR isolate (19), which were the least resistant uropathogens determined by this study.

Furthermore, in this study most of MDRE showed less susceptibility pattern to many of tested antibiotics. Particularly, resistance pattern were alarmingly higher for ampicillin (97.5%), cotrimoxazole (64.3%), chloramphenicol (61.2%), gentamycin (58.7%), ceftazidime (56.3%), amoxicillin-calvulanic (50.7%) and tetracycline (49.4%). Enterobacteriaceaeisolates were appeared about twice more resistant to cefpodoxime than to naldixicacid (44.4% vs 20%), and twice more susceptible to cefotaxime than amoxicillin-calvulanic (50% vs 24.4%) and gentamycin (50% vs 27.5%). On the other side, majority of MDR isolates showed susceptibility tociprofloxacin (90.6%), followed by cefepime (67.5%) and ceftriaxone (66.9%). Despite variation in proportion of resistant strains, similar circumstance of resistance pattern were also reported in Senegal, 77.3%, 55%, 14.7%, 13.3% of MDR isolates were resistant to ampicillin,

cotrimoxazole, amoxicillin-calvulanic acid and naldixic acid, respectively (22). As general point, most documented finding claimed that rate of antibiotic resistant drastically elevated, despite difference in magnitude of drug resistance. This might be attributed by policies on drug prescription, variability of guidelines for antibiotic prophylaxis or empiric treatment, variation in study population, geographical location, and socio-economical factors.

According to world health organization, 2014 report, in most of developed countries, the epidemiology of CPE isolates have frequently elucidated, whereas in others including African countries no sufficient epidemiological information is available, therefore the report insisted that integrated surveillance program and involvement of very active investigators have to be maximized in order to know the extent of resistant strainsin developing countries. Even though carbapenems drugs are not formally introduced in to Ethiopia, as the report claimed that increase international travel, globalization, migration, and medical tourism, might have contributing role in the dissemination of resistant strains from potentially risk countries (59). Particularly, in our study area, there is high tourist flow, andmany of residences have Diasporarelatives from abroad especially United States of America, which may have an impact on the emergence of carbapenemase producing, strains in this locality.

As revealed from the present study, out of 183 Enterobacteriaceae isolates, 2.73% (95% CI, 0.5 – 5.5%) were found to be carbapenemase producers among species isolated from patients with symptomatic UTIs. A comparable incidence of CPE were reported in studies Morocco (2.8%) (49), Bangladesh (4.8%) (43), Taiwan (2.5%) (46), Belgium (3.5%) (36), and India (5.4%) (40). while, other studies revealed that relatively high prevalence of CPE were investigated in Pakistan (8.6%) (42), Turkey (10.9%) (38), India (12.9%) (41), Nigeria (14%, 33.5%) (47, 48), Iran (14.5%) (45), and United States of America (21%) (39).

The difference in the incidence of CPE among published data and the present study might be due to trends in the utilization of carbapenems and other broad spectrum antibiotics, cultural/traditional relationships, exchange of population with other countries of high prevalence, cross boarder transfer of patients, travel, medical tourism, and refugees. Additionally, difference

in target study population, sample size variation, methodological variability could result variation in the epidemiology of CPE.

According to EARS surveillance study, many of carbapenemase producers were *K. pneumoniae* followed by *E.coli* and*Enterobacter* spp., the rest were other Enterobacteriaceae isolates(33). The same situations were also notified in finding from Turkey and Moroccoindicated that *K. pneumoniae* were the principal isolate followed by *E.coli and* K.*oxytoca* were important species of carbapenemase producer (38, 49). In spite of similarity among carbapenemase producer, but the proportion of isolates is incomparable to our study. We attested that 5 Enterobacteriaceae isolates were found to be carbapenemase producernamely;*2 E.coli, 2 K. pneumoniae*, and 1 *E. aerogenes*. On the other hand, a study from Nigeria demonstrated that *E.coli* was the main carbapenemase producer followed by *Proteus* spp. and *K. pneumoniae* (48). The variation among studies with regard to the proportion of carbapenemase producing isolates; could be due to difference in geographical distribution of isolates, target study population, sample size, and methodology used in each investigation.

Carbapenemase producing Enterobacteriaceae are extremely resistant to almost all available drugs in the market, including β-lactam and non β-lactam antibiotics (6), instantaneously this circumstance was also demonstrated in our study. Therefore, we tried to explore the antibiotic resistance pattern of CPE; hence all CPE isolates showed resistance to many of antibiotics, 100% resistance to cefotaxime, cefpodoxime, cotrimoxazole, chloramphenicol, ampicillin, and amoxicillin-calvulanic acid; 80% resistance to ceftazidime, and gentamycin; 60% resistance to ceftriaxone, cefepime, tetracycline, and naldixic acid. Interestingly, 60% of isolates were susceptible to ciprofloxacin, the reason mightbe due to carbapenemase producer are primary active against β-lactam drugs; even though, some of isolates co-expresses other resistance mechanisms to non β-lactam agents (quinolone, aminoglycosides).

In agreement to our study, finding from Kenya advocated that all carbapenemase producers were 100% resistant to b-lactam agents, non b-lactam agents (aminoglycosides, fluoroquinolones, chloramphenicol, sulfonamides) and including carbapenems (51). Similarly a study from Morocco also documented that the isolates were 100% resistant to amoxicillin-calvulanic acid,

cefotaxime , ceftriaxone, and cotrimoxazole, but gentamycin resistance decreased by half, and ciprofloxacin resistance increased almost by 2 times as compared to our finding(49). Those discrepancies might be variation in study population, standards employed for the interpretation of antimicrobial susceptibility testing.

In Bivariate analysis, age (years), hospitalization withinthe past 12 months, prior antibiotic therapy in the past 6 months, and prior UTI in the past 12 months were associated with MDRE UTI in this study. Likewise, in multivariable analysis, age, being female, hospitalization within the past 12 months, and prior antibiotic use in the past 6 months were the independent risk factors for MDRE UTIs. The same scenario also documented in a study in Chicago (16), showed that the above specified risk factors were also associated with MDRE UTIs. However, additional risk factors like health care associated risks (use of urinary catheter, mechanical ventilation, and hemodialysis) were identified in the former study, which were not indicated in this study. The reason could be due to variation in sample size, study design, geographical area and study population.

Moreover, this study also emphasized to assess risk factors associated with the colonization of CPE. However, none of the factors (age, sex, hospitalization, prior antibiotic use) were associated. This finding is completely disagree from result provided by several literatures, for instance based on ECDC report, advanced age, prior antibiotic use, hospitalization, chronic diseases (HIV/AIDS, diabetes mellitus), ICU admission and health care associated factors were associated with CPE infection (52). We suggested that such difference could be due to rare nature of the incidence and requires large scale study in order to point out risk factors. Besides, this is the first time investigation, and difficult to indicate all possible circumstances, which are directly or indirectly linked to CPE colonization. The present study only includes patients with symptomatic UTI; this is also the main thing for difficulty to pick up truly associated variables as mentioned by previous studies. Furthermore, study design variation may also attribute for this outcome presented by our study.

8. LIMITATION OF THE STUDY

In the present study, investigation of CPE was determined by using phenotypic methods, but could be better when it is supplemented by molecular techniques, which are essential to characterize the specific types of carbapenemase.

9. STRENGTH OF THE STUDY

This is a timely study, since antibiotic resistance becoming an alarming problem in both developed and developing world. Therefore, our study is perhaps the first one to identify new drug resistance mechanisms via the production of carbapenemase among Enterobacteriaceae uropathogens; which were not studied in our country at all.

10. CONCLUSION

The finding demonstrated that, high rates of drug resistance were observed among Enterobacteriaceae uropathogens, taking resistant two or more drugs; it was detected in 87.4% of isolates, *E. coli* and *K. pneumoniae* were the principal MDR isolates. Multidrug resistant Enterobacteriaceaeexhibited high drug resistance rates to ampicillin, followed by cotrimoxazole and chloramphenicol. Sex (female), age, hospitalization, and prior antibiotic use were independent risk factors for MDRE.Moreover, in the present study new drug resistance mechanisms via carbapenemase production among five Enterobacteriaceae isolates were detected. The isolates were completely resistant to ampicillin, cefotaxime, cefpodoxime, cotrimoxazole, chloramphenicol, and amoxicillin-calvulanic acid, only ciprofloxacin had showed low resistance rate to those strains, besides these isolates were found to be 100% ESBL producer.

11. RECOMMENDATIONS

Based on this finding the following recommendations are put forward to

Governmental bodies (hospital administrators)

1. Should take direct responsibility to facilitate periodic surveillance to control and prevent the emergence of MDR strains
2. Re-enforcement of local microbiology laboratories for the detection of CPE should be promotedthrough introduction of new methodologies.

Health professionals

1. Multidisciplinary approach is required in order to reduce patient hospital stay, and for appropriate prescription of antibiotics.
2. Should pay special attention to elderly and female patients, since those groups easily vulnerable to acquire infection caused by MDR strains

Researchers

1. Meticulous scientific and medical research, especially on CPE is so substantial, which includes using relatively standard methods, large study population and geographical area should be considered
2. Trends in the prevalence, and antibiotic resistance pattern of Enterobacteriaceaeshould continually reassessed through rigorous scholar findings

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13.ANNEXES

Annex I: QUESTIONNAIRE& LABORATORY DATA COLLECTION FORM

Interviewer administered questionnaire (to be filled by health professionals): Patient ID___

Aim of the study: to assess the prevalence and risk factors of multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infection.

Section 1: <u>DEMOGRAPHIC INFORMATION:</u>

S.no.	Question	Code	Response
1.	Sex	0 = Male, 1 = Female	
2.	Age	_____ years	
3.	Nationality	_____	
4.	Residence	1 = Urban 2 = Rural	
5.	Education status	0 = illiterate, 1 = Primary school, 2 = Secondary school, 3 = diploma & above	

Section 2: CLINICAL INFORMATION

S.no	Questions	Code	Response
1.	Sender of the patient	1 = outpatient , 2 = inpatient	
2.	If in patient , Name of admission ward: _____ Date of admission: _____		
3.	Admitted to this health care facility from	0 = home 1 = other hospital/health center	
4.	If the response is admission from other hospital/health center, name_____		
5.	Did you have a history of travel abroad in the last 12 months prior to hospitalization	0 = No 1 = yes	
6.	If yes: name of the country _____		

7.	State the reason for travel abroad	1 = holiday, 2 = work, 3 = medical intervention, 4 = visiting friends & relatives, 5 = other	

Section 3: RISK FACTOR FOR MDRE/CPE: please tick a code that apply to this patient

S.no	Questions	Code	Response
1.	Did you have history of hospitalization in the past 12 months	0 = No, 1= Yes	
2.	Did you have history of ICU admission in the past 12 months	0 = No, 1= Yes	
3.	Did you have history of UTI infection past 12 months	0 = No, 1= Yes	
4.	Did you have history of suffering with disease/infection condition (UTI, meningitis, pneumoniae, wound, bacteraemia, peritonitis, tuberculosis, GIT infection, and other infection mainly caused by bacteria) the past 12 months	0 = No 1= Yes	
5.	If yes, did you have history of antibiotic therapy for the specified disease/infectionsince the past 6 months	0 = No 1= Yes	
6.	Did you have history of using urinary catheter in the past 12 months	0 = No, 1= Yes	
7.	Did you have history of using mechanical ventilation/ breathing machines in the past 12 months	0 = No 1= Yes	
8.	Did you have history of surgery in the last 6 months	0 = No, 1= Yes	
9.	Are you pregnant	0 = No, 1= Yes	
10.	Did you have history of hemodialysis in the last 3 months	0 = No, 1= Yes	

Part II. LABORATORY DATA COLLECTION FORM

S.no	Question	Code	Response
1.	Does Enterobacteriaceaeisolated	0 = No, 1 = yes	
2.	Type of Enterobacteriaceaeisolated	1. *E. coli,* 2. *K. pneumoniae,* 3. *K. ozaenae,* 4. *K. oxytoca*, 5. *E. aerogenes,* 6. *E. cloacae*, 7. *P. mirabilis*, 8. *P.*vulgaris, 9. *Citrobacter* spp.	
3.	MDRE organism isolated	0 = No, 1 = Yes	
4.	CPE Organism isolated	0 = No, 1 = Yes	

5. Antibiotic Susceptibility Pattern:

Name of Antibiotics	Sensitivity Results (CLSI, 2013): Zone diameter breakpoints nearest to whole mm					
	Disc content	Susceptible (S)	Intermediate (I)	Resistant (R)	Zone Reading(mm)	RESULT (S or I or R)
Cefotaxime (CTX)	30µg					
Ceftazidime (CAZ)	30µg					
Ceftriaxone (CTR)	30µg					
Cefpodoxime (CPD)	30µg					
Cefepime (CPM)	30µg					
Ciprofloxacin (CIP)	5µg					
Tetracycline (TE)	30µg					
Trimethoprim-sulphamethaxzole (SXT)	25µg					
Chloramphenicol (C)	30µg					
Ampicillin (AMP)	10µg					
Naldixic acid (NA)	30µg					
Gentamycin (GEN)	10µg					
Amoxicillin-calvulanic acid (AMC)	30µg					

Annex II: Urine collection and processing

Specimen collection: First morning specimens yield highest bacterial counts from overnight incubation in the bladder, and are the best specimens.

Procedure for midstream urine for bacterial investigation

1. Give the patient suitable container
2. Instruct the patient before the collection, preferably with illustration.
3. Tell the patient not to touch the inside or rim of the container

- Male
  - If not circumcised, draw back the foreskin
  - Begin to urinate, but pass the first portion into the toilet.
  - Collect the mid-portion of urine into the container, and pass the excess into the toilet.
- Female
  - Squat over the toilet and separate the labia with one hand.
  - Void the first portion of urine into the toilet.
  - Collect the mid-portion of urine into the container and pass the excess into the toilet.
- Infants:Have ready: Clean, preferably sterile container of appropriate size or aplastic bag, cotton wool or gauze pads, hand warm soapy water.
  - Clean the external genitals.
  - Give the child as much liquid as possible just prior to the collection
  - Seat the child on the lap of the mother, nurse or ward attendant
  - Collect as much urine as possible in the container or plastic bag when theinfant urinates.

Processing: approximately 20ml of urine sample is required; the maximum time allowed for processing a urine sample is 2 hours from the time of collection. If delay is inevitable, should be refrigerated / preserved (boric acid). During culturing, the urine must be re-suspended and streak 1µl of the volume to blood agar. After overnight incubation plate count of 100,000 CFU/ml of pure culture should be considered positive and isolated organism should be identified and sensitivity test will be performed.

Annex III: urine culture for isolation of MDR &CPE

Urine culture on blood agar plate

- Dips the 0.001 ml loop into the urine same (just into the urine, without submerging the plastic sample).
- Place a straight line down the center of the agar plate, then streak in a dense zigzag pattern back and forth across the plate to the bottom.
- Overnight Incubation at 37^{o}c
- Check for growth, calculate the number of bacteria in the urine sample = no of colony count x dilution of calibrated loop (1000)
- If it is $\geq 10^{5}$/ml of urine, gram staining will be done to decide the predominant colony type (gram –ve or gram +ve bacteria)

MacConkey agar for gram –ve rod bacteria

- Sub-culture pure colonies from BAP to MAC.
- Overnight Incubation at 37^{o}c
- Check for growth, and lactose fermentation
- Perform biochemical test for Enterobacteriaceae identification

Biochemical tests for Enterobacteriaceae

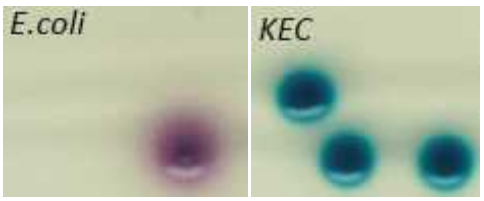
Isolate	TSI	Gas prod.	H2S prod.	Indole	citrate	urea	motility	Lysine dec
E.coli	A/A	+	-	+	-	-	+	+
Citrobacter spp	A/A	+	+	-	+	+/-	+	-
K. pneumoniae	A/A	+	-	-	+	+	-	+
K. oxytoca	A/A	+	-	+	+	+	-	+
E. aerogenes	A/A	+	-	-	+	-	+	+
E. cloacae	A/A	+	-	-	+	+/-	+	-
P. mirabilis	Ak/A	+	+	-	+/-	+	+++	-
P. vulgaris	A/A	+/-	+	+	-/+	+	+++	_

Antimicrobial susceptibility testing on Muller Hinton agar

- Take 3 – 5 pure colonies of the isolate and suspend in nutrient broth
- Adjust the turbidity of the broth with 0.5% barium sulphate solution
- Dip a sterile cotton swab into the broth suspension. Rotate the swab several times on the inside wall of the tube above the fluid level to remove excess inoculum from the swab
- Inoculate the surface of the plate by streaking the swab over the surface of the plate 2/more times.
- Place the appropriate discs onto the respective cultures. Deposit discs so that the centers are at least 24 mm apart.
- After overnight incubation, measure zone of inhibition to the nearest mm. report as resistant, intermediate, & susceptible according to CLSI guidelines

Isolation of CPE using CHROMagar KPC medium

- Pure colonies of MDRE isolates will be inoculated to the agar plate
- After overnight incubation, CPE will produce colonies with typical coloring features.



Quality control strains
K. pneumoniae ATCC 1705 ------- steel blue
S. aureus ATCC 25293--------- inhibited

Annex IV: patient information sheet and consent form

Patient information sheet

Study title:	Prevalence and risk factors of multidrug resistantand carbapenemase producing Enterobacteriaceaeamong patients with urinary tract infection, Gondar University Hospital, North west Ethiopia.		
Locality:	University of Gondar: department of Medical Microbiology	Ethics committee:	UOG
Lead investigator:	Setegn Eshetie(BSc.)	Contact phone number:	0913151163

You are invited to take part as a study participant in the research conducted by MSc candidate in University of Gondar. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason, and it won't affect the care you receive. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep. This document is 3 pages long, including the Consent Form. Please make sure you have read and understood all the pages.

Purpose of the study: emergence of drug resistance among bacteria, especially in Enterobacteriaceae is a major public health treat. Knowledge about current prevalence of

carbapenemase producing and MDRE, associated risk factors is so substantial to design possible intervention and preventive strategies. Hence the aim of this project is to explore prevalence and risk factors ofMDREand carbapenemase producing strains.

Procedure: In order to perform the indicated study at Gondar university hospital you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent. The required clinical sample will be collected by you.For severely ill patients, infants, the sample collection may aided by health care providers/ families.

Possible risks/discomfort: There are no anticipated risks to your participation. Sample collection for severely ill patients may require urinary catheter, in such procedure you may feel some discomfort. But this not predisposes you to unwanted problems.

Benefits of study: you will be treated accordingly as result of diagnosis procedure. Instantaneously; after completion of this study, it provides millstone information to design preventive and control measures so that the society will be benefited from the outcome of study.

Compensation for participation: You will not receive any payment for your participation in this research study.

Confidentiality: There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential. The information collected about you will be coded using numbers.

Participation and withdrawal: You can choose whether to be a part of this study or not. You may withdrawal at any time without consequences of any kind. You may also refuse to give any sample.

Person to contact: If you have any question you can contact any of the following (Investigator and Advisors) and you may ask at any time you want.

Setegn Eshetie: Cell phone: 0913151163, E-mail: seteeshetu@yahoo.com, Face book: Sete Eshetu

Patient consent form (≥ 18 years of age)&able to respond

I have read, or have had this document read to me in a language that I understand, and I understand the purposes, procedures and risks of this research project as described within it.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project, as described.

I understand that I will be given a signed copy of this document to keep.

Participant's name ……

Signature Date

For families or attendants of patients unable to respond

I_____ parent/guardian/attendant, after being fully Informed about the purpose of this study, hereby give my consent on the patient's Participation in this study. I understand that my child free to withdraw at any time without penalty or loss of benefits.

Signature of first parent or guardian _____ date _____

Declaration: I have given a verbal explanation of the research project, its procedures, other related issues and I believe that the participant has understood that explanation.

Researcher's name: Setegn Eshetie

Signature Date

አማረኛ ትርጉም (version)

መድሀኒት መቋቋም የሚችሉና በተለይም ካርፓፔንሜዝ የሚባል እንዛይም የሚፈጥሩ ተዋስያንበማስመልከት፣ በተጨማሪም የተለያዩ የሚያጋልጡ ሁኔታዎችን ለማጥናት በሽንት ፊኛ ታካሚዎች ላይ የተዘጋጀ መጠይቅ ነው።።

መመሪያ (በጤና ባለሞያዎች የሚሞላ): እባክዎን ከዚህ በታች ለተዘረዘሩ ጥያቄዎች ከፊት ለፊት ከተቀመጡት ምርጫወች ታካሚውን በመጠየቅ፤ በመመልከት እና ሌሎች መረጃዎችን በመውሰድ (medical record, clinical & laboratory diagnosis)በተዘጋጀው ሳጥን ምልክት በማድረግ እና ክፍት ቦታወችን በፅሁፍ በመግለጥ መልስ ይስጡ::

ክፍል አንድ:አጠቃላይ ማህበራዊ ነክ መረጃዎችን ለመዳሰስ የተዘጋጀ።። የታካሚው ኮድ---

ተ/ቁ	ጥያቄ	ኮድ	መልስ
1.	ፆታ	1 = ወንድ፣ 2 = ሴት	
2.	እድሜ፡	----------አመት	
3.	ዜግነት፡	----------	
4.	መኖሪያ በታ	1 = ከተማ ፣ 2 = ገጠር	
5.	የት/ት ሁኔታ	0= ያልተማረ፣ 1 =1ኛ ደረጃ ያጠናቀቀ፡ 2 = 2ኛ ደረጃ ያጠናቀቀ፣ 3 = ከዚያ በላይ	

ክፍል ሁለት፡ አጠቃላይ የህክምና መረጃዎችን እና ሌሎች ተዘማጅ ሁኔታዎች ለመዳሰስ የተዘጋጀ

1.	የሕክምና ሁኔታ	1 =ተኝቶ የሚታከም፤ 2=ተመላላሽ	
2.	ተኝቶ የሚታከም/የምትታከም/ ከሆነ	የዋርዱ ስም ----------የተኛበት/የተኛችበት/ ቀን----------	
3.	ወደ ሆስፒታሉ የመጡት ከሌላ ጤና ድርጅት ሲታከሙ ቆይተው ነው	1 = አዎ፡ 2 = አይደለም	
4.	ከአንድ አመት በፊት ወደ ሌላ ሀገር ሂደው ያወቃሉ	1 = አዎ፡ 2 = አይደለም	

5.	አወ ካለ የት ሀገር	----------	
6.	የሄዱበት ምክንያት ለምንድን ነው፡	1 = ዘመድ ለመጠየቅ፤2 = ለህክምና፡ 3=ለጉብኝት፤ 4 =ለስራ፤ 5 =ለበአል ፤6 =ሌላ ካለ ይጥቀሱ	
ክፍል 3፡ ከዚህ በታች የተዘረዘሩት ሁኔታዎች ታካሚውን የሚጠቅስ ከሆኑ ከፊት ለፊት በተዘጋጀው ሳጥን ምልክት			
1.	ከዚህ በፍት በ ጤና ድርጅት ውስጥ ተኝተው ታክመው ያውቃለ	1 =አዎ፤ 2 = አይደለም	
2.	ከዚህ በፊት ህይወትዎን ለአደጋ የሚጥል አደጋ/በሽታ ተጋልጠውና ታክመው ያውቃለ	1 =አዎ፣ 2 = አይደለም	
3.	የቀዶ ጥገና ህክምና ተደርጎልት ያወቃል ወይ	1 =አዎ፣ 2 = አይደለም	
4.	በሽተኛው ኤች አይቪ/ኤድስ በደሙ/ሚ ዉስጥ ተገኝቷል ወይ (በጤና ባለሙያ የሚመለስ)	1 =አዎ፣ 2 = አይደለም	
5.	የወለድ መቆጣጠሪያ ተጠቅመው ያውቃለ	1 =አዎ፣ 2 = አይደለም	
6.	የስኳር በሽታ ተጠቂ ነዎት ወይ (በህክምና ምርመራ ወይም በተጠያቂው የሚመለስ)	1 =አዎ፣ 2 = አይደለም	
7.	ነፍሰ ጡር ነዎት ወይ (በህክምና ምርመራ ወይም በተጠያቂው የሚመለስ)	1 =አዎ፣ 2 = አይደለም	
8.	ከ አሁን በፊት የተለያዩ መድኒቶችን ለፀረ-ተዋህሲያን ተጠቅመው ያውቃለ	1 =አዎ፣ 2 = አይደለም	
9.	ከአሁን በፊት የሽት ፊኛ ህመም ታመው ያውቃለ	1 =አዎ፣ 2 = አይደለም	
10.	የሽንት መሸኛ ትቦ ተደርጎወለት ያውቃል ወይ	1 =አዎ፣ 2 = አይደለም	
11.	የአየር መተንፈሻመሳሪያ ተደርጎዎለት ያወቃል	1 =አዎ፣ 2 = አይደለም	
12.	የደም ማጥራት ህክምና ተደርጎዎለት ያወቃል ወይ	1 =አዎ፣ 2 = አይደለም	
13.	ከላይ የተጠቀሱት አጋላጭ ሁኔታዎች የለብኝም	1 =አዎ፣ 2 = አይደለም	

የጥናቱ ማብራሪያና ስምምነት ቅጽ

1. ጥናቱን የሚያካሂደው ሰው ስም

ሰጠኝ እሸቴ (የመጀመሪያ ዲግሪ ምሩቅና በጎንደር ዩንቨርስቲ የሁለተኛ ዲግሪ ተማሪ)

2. የጥናቱ ዓላማ

ጥናቱ የሚካሄደዉ የሽንት ፊኛ ህመም ተጠቂ በሆኑና በጎንደር ዩንቨርስቲ ሆስፒታልተገኝተዉ በሚከሙበሽተኞች ላይ ሲሆን ዓላማዉ መድሀኒት የመቋቋም ብቃት ያላቸውን ባክቴሪያዎችንመለየትናበተለይም ካርፓፔንሜዝ የሚባል እንዛይም በሚፈጥሩ ተዋስያንበማስመልከት; እና ተዛማጅ አጋላጭ ምንገዶችን ለይቶ ለማውጣት ነው፡፡

3. በጥናቱ ስለመሳተፍ

በዚህ ጥናት መሳተፍ በሙሉ ፈቃዳኝነት ላይ የተመሰረተ ነዉ፡፡ ስለሆነም በጥናቱ አንዲሳተፉ ፈቃደኝነትዎን እንጠይቃለን፡፡ በጥናቱ ሊሳተፉ ከተስማሙ ሐኪሙ ወይም ዋናዉ ተመራማሪ ለሚጠይቅዎት ጥያቄዎች መልስ መስጠት ይጠበቅብዎታል፡፡ ለምርመራ የሚሆን ሽንትና ደም ኢንዲወሰድ ፈቃደዎን እንጠይቃለን፡፡

4. ሊከሰቱ የሚችሉ ስጋቶች

ለጥናቱ በሚወሰድ ናሙና ምክንያት የተለየ ችግር አይከሰትብዎትም፡፡ የናሙና አወሳሰዱ በሽተኛዉ ለራሱ ብሎ ከሚሰጠዉ ናሙና የተለየ አይደለም፡፡ ሽንት መሽናት ለሚያቅተቸውታካሚዎች ናሙናው በቱቦ በሚወሰድበት ጊዜአንደማንኛዉም በሽተኛ የተለመደ ቲንሽ አለመመቻቸት ለተወሰነ ደቂቃ ሊኖር ይችላል፡፡

5. በጥናቱ በመሳተፍ የሚገኝ ጥቅም

በሽተኞች ከምርመራዉ ዉጤት ቀጥተኛ ተጠቃሚ ይሆናሉ፡፡ የምርመራዉን ዉጤት ከሀኪሞች ጋር በመነጋገር በአጠቃላይ ለሕመምተኞች በሆስፒተሉ የተሻለ ፈጣን አንክብካቤ ለማድረግ ያግዛል፡፡

6. ክፍያን በተመለከተ

በዚህ ጥናት በመሳተፍዎ ምንም አይነት ክፍያ አይከፈለዎትም፡፡

7. የጥናቱ መረጃዎች ሚስጢራዊነት

በጥናቱ ዉስጥ የተሰበሰቡ ማናቸዉም ግላዊ መረጃዎች ሚስጢራዊነት የተጠበቀ ይሆናል፡፡ ከማንነት ጋር በቀጥታ ተያያዥነት ያላቸዉ መረጃዎች በሙሉ በዋና ተመራማሪዉ ሚስጢራዊ በሆነ የመረጃ ጥንቅር ዘዴ ከተቀየሩ በኋላ ለምርምር ሂደቱ ብቻ የሚዉሉ የሆናሉ፡፡

8. ከጥናቱ ስለመዉጣትና ስለማቋረጥ

ይህ ጥናት በፍቃደኝነት ላይ የተመሰረተ አንደመሆኑ መጠን በማንኛዉም ወቅት በፈቃድዎ ከጥናቱ መዉጣት ይችላሉ፡፡ከጥናቱ ቢወጡም የተለመደዉን የሕክምና እርዳታበጤና ተቋሙ

ወስጥ በማንኛውም ጊዜ የማግኘት መብት አልዎት::

9. ከትናቱ ጋር በተያያዘ ማናቸውም ጥያቄ ቢኖር– በሚከተለዉ አድራሻ ጥያቄዎን ማቅረብ ይችላሉ፡-
   - ሰጠኝ እሸቴ፡ አድራሻ፡- ጎንደር ዩኒቨርሲቲ ሕክምናጤና ሳይንስ ፋካልቲ፤ ስልክ 0913-151163፤ ኢ-ሜይል፡ seteeshetu@yahoo.com

የስምምነት መግለጫ ቅጽ

የበሽተኛዉ ምስጢር ቁጥር----------
የበሽተኛዉ ሙሉ ስም----------

አኔ ስሜ ከዚህ በዚች የተገለጸዉ በዚህ ጥናት ተሳታፊ ለመሆን ስወስን የጥናቱ ዓላማዎች አሰራሮችናቅድመ ሁኔዎች በግልጽ በመረዳትና እንዲሁም ከጥናቱ ተሳተፊነት ፈቃደኝቴን በማንኛዉም ደረጃየማስወገድ መብቴን በማረጋገጥ ነዉ::

በዚህጥናትተሳተፊ መሆኔን በፊርማየ እያረጋገጥኩ ይህንን ስወስን በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ ስጋቶችን በሚገባ የተረዳሁና ከጥናቱ በማንኛዉም ደረጃ ራሴን ለማግለል ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉእንደማይነፈጉኝ በማመን ነዉ:: እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጹልኝ መሆኑን በፊርማዬ አረጋግጣለሁ::

የበሽተኛዉ ሙሉ ስም---------- ፊርማ----------

የተመራማሪዉ ሙሉ ስም---------- ፊርማ----------

የምስክር ሙሉ ስም----------ፊርማ----------ቀን፡ -----/-----/-----

ለሕፃናትና ሀሳባቸውን መግለፅ ለማይችሉ አዋቂዎች አስታማሚዎች

እኔ _____ የበሽተኛው አስታማሚ ስሆን የዚህን ጥናት ዓላማ በውል በመገንዘብ በሽተኛው በጥናቱ እንዲሳተፍ የምስማማ መሆኔን በፊርማዬ አረጋግጣለሁ::

ፊርማ ----------ቀን፡ -----/-----/-----

DECLARATION

The thesis paper is our original work, has not been presented for a degree in any other University and that all sources of materials used for the thesis paperhas been duly acknowledged.

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